Practical lesson 7
Fundamentals of antimicrobial therapy. Chemotherapeutic drugs. Antibiotics, their production and classification. Evaluation of the sensitivity of microbes to antibiotics.

- ***Fundamentals of chemical therapy***

- Treatment of infectious diseases with chemical therapeutic drugs is called chemical therapy .These drugs do not treat symptoms. They have effect on etiological pathogens. Thus, they are called etiotropic drugs. Nowadays, thousands of chemical compounds with antimicrobial activity is known. However, only some of them are used as chemotherapeutic drugs. Activity spectrum of therapeutic drugs is determined by microorganism groups on which they have effect. Based on type of affected microorganism antibacterial, antifungal, antiprotozoan, antiviral etc., antimicrobial drugs exist.

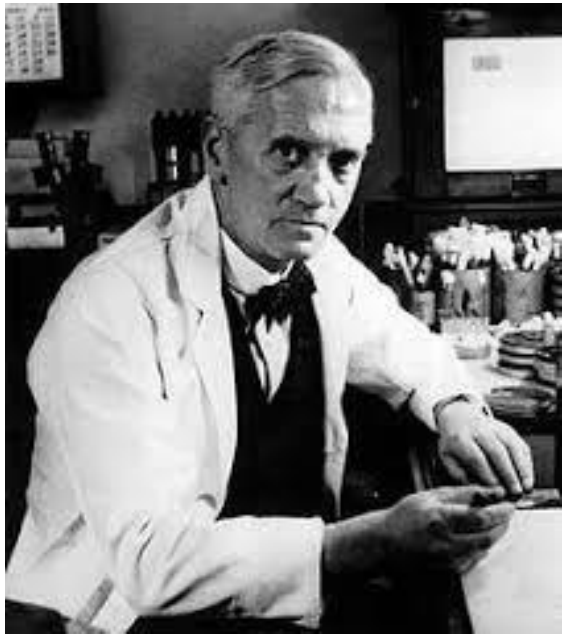
- *Paul Ehrlich is the founder of chemotherapy* . In1885 P.Ehrlich discovered that the impact of chemical compounds on pathogens is related to specific receptors in microorganisms. “Magic bullet” idea of P.Ehrlich was one of the main principles of chemical therapy. It proposed killing microbe without impact on body tissues. Chemical therapeutic index – ratio of minimal therapeutic dose killing pathogen to the highest dose which organism can resist. This index is used for evaluation of therapeutic drugs. Nowadays, thousands of chemical compounds with antimicrobial activity is known. However, only some of them are used as chemotherapeutic drugs. Activity spectrum of therapeutic drugs is determined by microorganism groups on which they have effect. Based on type of affected microorganism antibacterial, antifungal, antiprotozoan, antiviral etc., antimicrobial drugs exist.

- Depending on activity spectrum narrow and broad spectrum drugs distinguished. *Narrow spectrum* drugs act on limited number of species, either gram negative or gram positive bacteria. *Broad spectrum* drugs both on gram negative and gram positive bacteria species.

- Based on action type: *Microbocide* (bactericide, fungicide etc.,) and *microbostatic* (bacteriostatic, fungistatic etc.,) drugs distinguished. The first group drugs kill bacteria, while representatives of the second group inhibit microbial growth.

- Based on method used to obtain antimicrobial drugs they are divided to: *synthetic* – commonly obtained by chemical synthesis; *antibiotics*-commonly of natural origin, sometimes obtained by syntetic or semisynthetic methods.

- ***The main groups of synthetic chemotherapeutic drugs***
- *Sulfanilamide* (streptocide, sulfadimidine, sulfadimetoxin etc.)
- *Antimetabolites* – nicotinic acid derivatives (isoniazid, ftivazid, tubazid etc.)
- *Quinolons* - nalidixic acid(nevigramon), ofloxacin, ciprofloxacin, norfloxacin etc.,
- *Nitroimidazoles* (metronidazole, ornidazole etc.)
- *8- oxyquinoline* (5-nitroксolin, xinozol, intestopan etc.)
- *Nitrofurantoin derivatives* (furacillin, furazolidone, furagin etc.)
- *Imidazoles* (кетоконazole, миконazole, clotrimazole etc.)
- *Triazoles*(флуконazole)



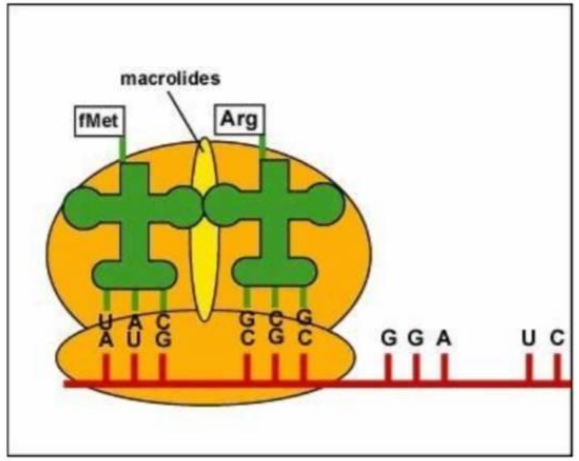
- ***Antibiotics***
- Production of antibiotics (greek anti – against, bios – life) by microorganisms is the common type of antagonism. Small concentrations of these compounds stop growth of other microorganisms. The term “antibiotic” was proposed by S. Vaxman in 1942. According to him antibiotics are compounds produced by microorganisms and stop the growth of certain bacteria or cause their destruction. In 1929 Alexander Fleming noted a lysis of Staphylococcus aureus colonies surrounding the culture of mould (Penisillium notatum) occasionally contaminated the Petri dish.
- ***Obtaining of antibiotics***
- They are excreted by microorganisms in nutrition media during growth of microorgansims and separated from media chemically. Some antibiotics are obtained by semisynthetic and synthetic methods. Thus, 3 main methods of antibiotic obtaining exist: biosynthetic, semisynthetic, chemicalsynthes

- **Classification of antibiotics**
- **Origin:**
- Antibiotics of microbial origin:
- - Bacteria(polymixin, qramicidin etc.)
- - Actynomicetes(streptomycin, tetracycline, choramphenicol etc.);
- - fungi synthesized antibiotics (penicillins, scfalosporins etc.);
- - Plant (phytoncides) Animal(lisozyme, interferone

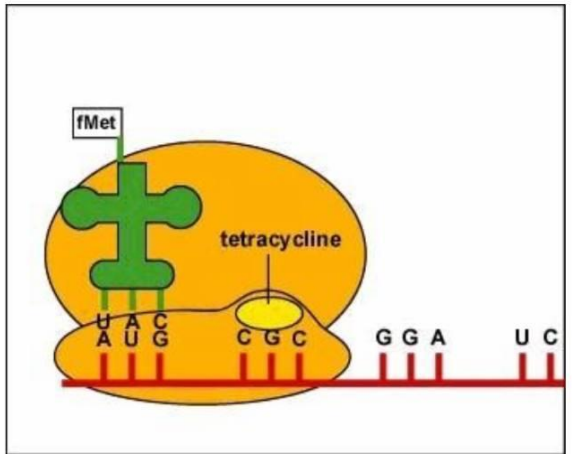
- **Chemical structure :**
- **beta-lactams** (penicillins, cefalosporins, carbapenems, monobactams)
- **macrolides** (erythromycin, spiramycin, кlaritromycin etc.)
- **azalides**(azitromycin) tetracyclines (tetracycline, doxycycline)
- **aminoglicosides**(streptomycin, кanamycin, gentamicin)
- **levomycetin(chloramphenicol)**
- **glikopeptides**(vanкomycin)
- **Rifamycins**(rifampin)
- **cyclic polipeptides(**polymixins, bacitracins)
- **polyenes**(nistatin, levorin, amphotericin B etc.)

- **Mechanism of action of antibiotics**
- *Inhibitors of cell wall synthesis* (antipeptidoglycan antibiotics). Betalactams(penicillins and cefalosporins), glycopeptides (vankomycin and teicoplanin)
- *Inhibitors of protein synthesis* (antiribosomal antibiotics) Aminoglycosides and tetracyclines act on 30S-subunit, macrolides, chloramphenicol and lincosamids – on 50S-subunit of ribosoms, resulting in stop of protein synthesis.
- *Inhibitors of nucleic acid synthesis* - rifamycins (rifampicin) bind to RNApolimeraze and block transcription – mRNA synthesis. Anribiotics altering cytoplasmic membrane permeability (membranotrope antibiotics) - polipeptides (polymixins), polyenes (nistatin, levorine, amphotericin
- Antibiotics that affect the permeability of the cytoplasmic membrane (membrane-anthropic antibiotics) - polypeptides (polymyxins), polyene antibiotics (nystatin, levorin, amorphous).

Macrolides act on 50S-subunit of ribosomes and stop protein synthesis



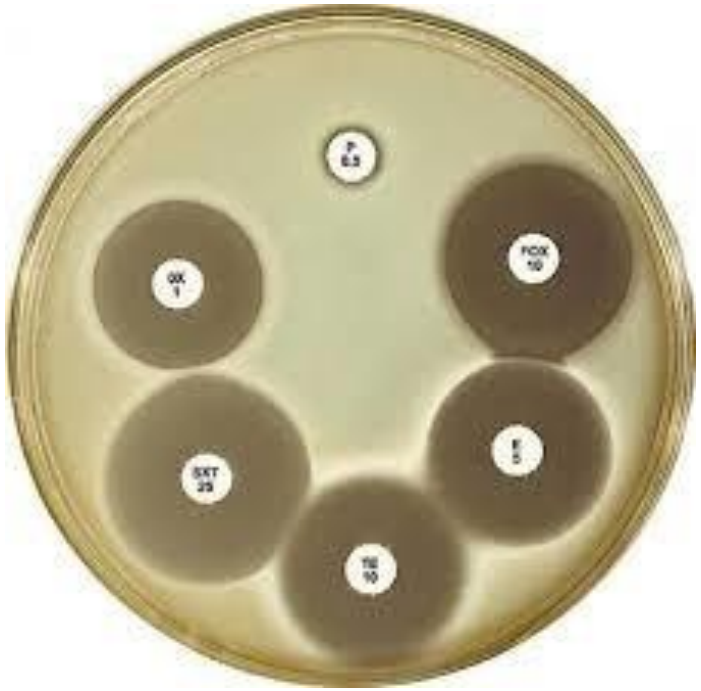
Tetracyclines act on 30S-subunit of ribosomes and stop protein synthesis



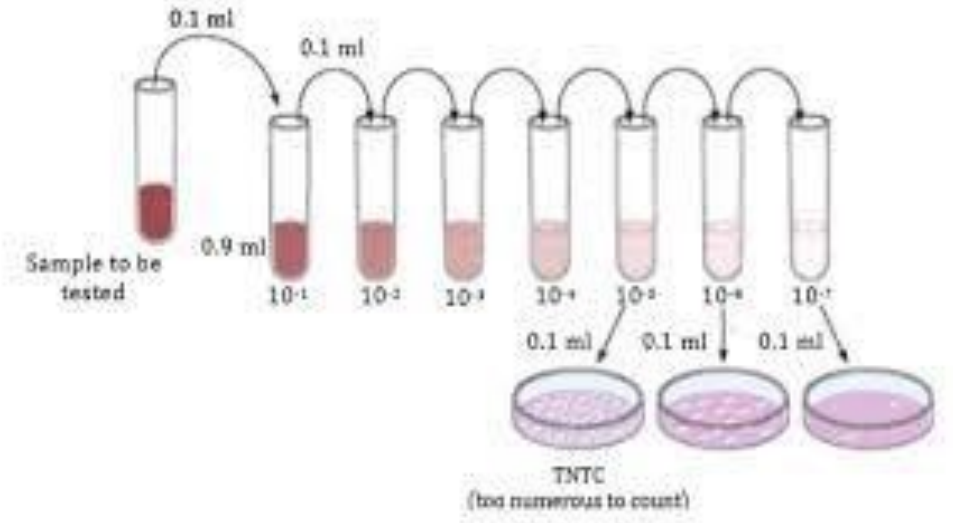
- **Antibiotic resistance of microorganisms and its mechanisms**
- Resistance to antibiotics is of two types, natural and acquired. Natural sustainability is related to the structural and biological properties of microorganisms. Acquired sustainability is associated with the adaptation of microorganisms to the external environment and occurs as a result of the action of antibiotics.
- - Decreased permeability of the cell wall to the antimicrobial agent and impairment of its perception of intracellular targets
- - Accelerate the removal of the antimicrobial agent from the cell.
- - Modification of the target of antimicrobial action - Inactivation of antimicrobial agent
- Decreased permeability of the cell wall to the antimicrobial agent and disruption of its transport to intracellular targets :
- The entry of drugs into the microorganism depends on the nature of the cell wall. Changes in the structure of pores under the influence of various factors, for example, mutagenic factors, are accompanied by a decrease in their permeability. R-forms, which are devoid of polysaccharide capsules and have relatively low levels of lipopolysaccharide, are sensitive to most antibiotics.
- *Modification of the target of antimicrobial action :*
- • Methylation of RNA (ribosomal RNA) in the 50S subcomponent of ribosomes is one of the main mechanisms of resistance to macrolides and linosamides.
- • Metallization of the nucleotide of only two adenes prevents the combination of these antibiotics with the 50S-components. The synthesis of the enzyme that catalyzes this process - methylase - is encoded in the R-plasmid.
- *Inactivation of an antimicrobial agent:* It is one of the main mechanisms of drug resistance of microorganisms. Some bacteria have the ability to synthesize special enzymes that inactivate antibiotics. Beta-lactamase (penicillin), a beta-lactamase (penicillin) that breaks down the beta-lactam ring in penicillins and cephalosporins to form inactive compounds between these enzymes. Synthesis of beta-lactamasesis encoded in R-plasmid.
- *Genetic basis of antibiotic resistance:* Resistance to antibiotics is mainly provided by resistance genes (r-genes). Plasmids that have resistance genes are called R-plasmids, or R-factor. Resistance genes primarily encode the synthesis of enzymes (eg, betalactamase, etc.) that ensure the drug resistance of microorganisms. Antibiotics do not induce the formation of r-genes, but only cause the selection of microbial populations that possess these genes.Mutations in the microbial population also play a role in ensuring the resistance of microorganismsto antibiotics. For example, the persistence of some S. aureus strains to methylcellulose is due to gene mutations in them that result in penicillin binding proteins, which is unable to bind to betalactam antibiotics. For this reason, methicillin-resistant S. aureus (MRCA) strains are resistant to all beta-lactam antibiotics.

- **Prevention of antimicrobial resistance**
- Ways to prevent resistance to antibiotics:
- • Rational use of antimicrobials
- • synthesis of new antibiotics
- • Combination of some antibiotics with betalactamase enzyme inhibitors (sulbactam and clavulanic acid):
- - The beta-lactam ring in these substances combines with beta-lactamases to neutralize them, thereby counteracting the effect of these enzymes on betalactam antibiotics.
- - Preparations of ampicillin combined with sulbactam (ampicid, etc.) and amoxicillin with clavulanic acid (augmentin, amoxiclav, etc.) are widely used in medical practice.
- One of the ways of antibiotic resistance prevention is evaluation of antibiotic susceptibility of microorganisms. Qualitative and quantitative methods for antibiotic susceptibility testing exist. *Qualitative method*. Disk-diffusion (Kirby-Bauer) is commonly used method.
- *Quantitative method*. Enables determination of minimal inhibitory and bactericide concentration of antibiotics.
- **Disk-diffusion** (Kirby-Bauer) is commonly applied method. For this purpose paper disks with impregnated antibiotics(with determined concentration) are applied to solid media with inoculated culture of microorganism. Disks (no more than 6) are placed on 90-mm Petri dishes. Antibiotic susceptibility is evaluated on basis of growth of microorganisms around the disks after 1-day incubation. Antibiotic susceptible bacteria do not grow around disks and sterile zones of various diameter are observed. The diameter of sterile zone depends on susceptibility degree of microorganism.

Paper disks with impregnated antibiotics

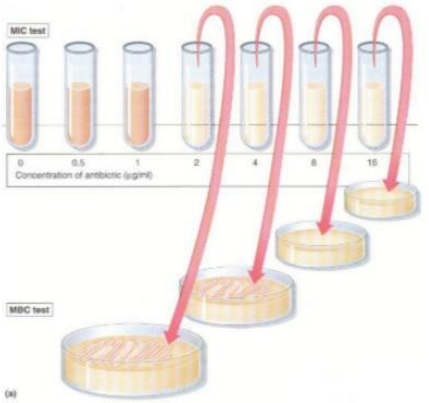


- **Quantitative method** makes possible detection of minimal inhibitory concentration (MIC) of antibiotics. The principle of method is based on growth of microorganism in nutrition media with added antibiotics of different concentrations. The lowest concentration of antibiotic inhibiting growth of microorganism is considered as minimal inhibitory concentration (MIC), the lowest concentration killing microorganism – minimal bactericide(microbocide) concentration (MBC or MMC). These values are interpreted in mcg/ml. For some antibiotics international units are used. Unit of antibiotic is the lowest dose inhibiting growth of microorganism. Commonly 1 IU is equal to 1 mcg.
- ***Serial dilutions method*** : By serial dilutions method minimal concentration of antibiotic inhibiting growth of microorganism is detected. For example, in order to detect MIC of tetracycline for Staphylococcus aureus double lethal concentration of this antibiotic is added to test tubes with nutrient broth. Content of first tube is added to 2nd , content of 2nd - to 3rd and so on. As a result series of diluted concentrations are obtained.

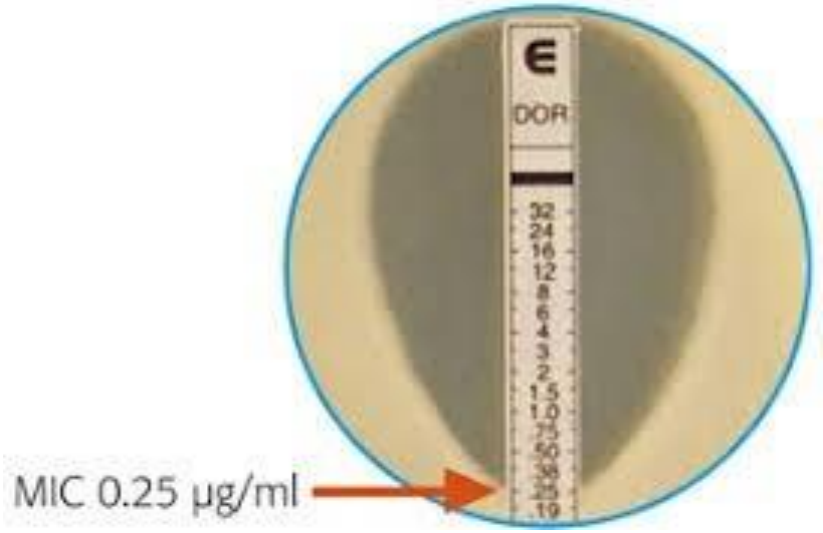


Detection of MIC by serial dilution method.

The principle of method based on growth inhibition of microorganism by different concentrations of antibiotics added to nutrient broth.



- **E-test method** is based on usage of paper strips with impregnated gradient of antibiotic concentration (128, 64, 32, 16, 8, 4, ..., mcq/ml). These strips are applied on nutrient media with inoculated culture of microorganism (as in disk diffusion). After incubation, bacterial growth becomes visible, symmetrical inhibition ellipse along the strip is seen. The MIC value is read from the scale in terms of µg/ml where the ellipse edge intersects the strip.



- ***Investigation of antibiotic resistance of microorganisms.*** Production of enzymes breaking down antibiotics is one of the mechanisms responsible for antimicrobial resistance. Beta-lactamases that break down the beta-lactam ring in beta-lactam antibiotics and inactivate them are one of the examples of such enzymes. Production of these enzymes are encoded by plasmid genes. Recently, the number of microorganisms that synthesize extended-spectrum beta-lactamase (ESBL) is increasing. Unlike conventional beta-lactamases, ESBL breaks down antibiotics that are resistant to beta-lactamases causing resistance to them.
- *Detection of ESBL synthesis in microorganisms (phenotypic test)* : Two disks – beta-lactame (exp. Cefipime) disk and antibiotic+betalactamase inhibitor (exp. Amoxiclav) containing disk are placed in nutrient agar with inoculated culture. Result is interpreted after 1-day incubation. In case of ESBL synthesis enhancement of sterile zone(in amoxiclav direction) around cefipime is observed.
- *Complications that can occur under the influence of antibiotics and ways to prevent them:*
- • Hypersensitivity reactions - allergic reactions- consideration of hypersensitivity reactions
- • Dysbiosis and dysbacteriosis
- - Combination of antibiotics with antifungal drugs during long-term use- use of eubiotics
- - representatives of normal microflora during long-term use
- Toxic effects - consideration of contraindications and side effects